Energy Landscape of Biotin-Streptavidin Interactions Selectively Probed using Dynamic Force Spectroscopy

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Dynamic Force Spectroscopy (DFS) has enabled us to investigate the specific interactions between two molecules, such as ligand-recepter pairs [1]. In DFS measurement, the unbinding force applied to a molecular bonding is increased at a constant rate, and the force required to rupture the molecular pair is measured. The energy landscape of the interactions is derived from the relationship between the rupture force and the loading rate.

However, the constant loading rate required for DFS experiment has not been realized because the force probe, in general, is retracted from the substrate at a constant velocity instead of constant loading rate, where it is difficult to analyze the landscape in detail. In order to achieve precise measurement, we have developed a system that has a feedback loop to keep the loading rate constant. The unbinding force applied to a molecular pair is detected using Atomic Force Microscopy, and the probe-substrate distance is precisely controlled with the feedback [2].

Here, we demonstrate the results of the selective analysis of molecular interactions obtained using this method for the Biotin-Streptavidin molecular system.

Figure 1 shows the most frequent rupture forces plotted against the logarithmic form of the loading rate, where streptavidin molecules were fixed on a Au substrate with two different forms as schematically illustrated. The potential-barrier position in the energy landscape is estimated from the slope of the linear relationship between the rupture force and the loading rate. For the case (a), the potential barrier position was estimated to be 0.68 nm, which is attributed to a transient network of water bridges and hydrogen bonds. In contrast, for the case of the type (b), there are two slopes that provide the potential barriers at 0.16 nm and 0.63 nm. Namely, an inner barrier due to direct hydrogen bond between biotin and amino acid in streptavidin was additionally probed. Such a detail analysis has become possible because the precise measurement, particularly at high loading rates, was achieved by the new method.

Details will be discussed at the conference.

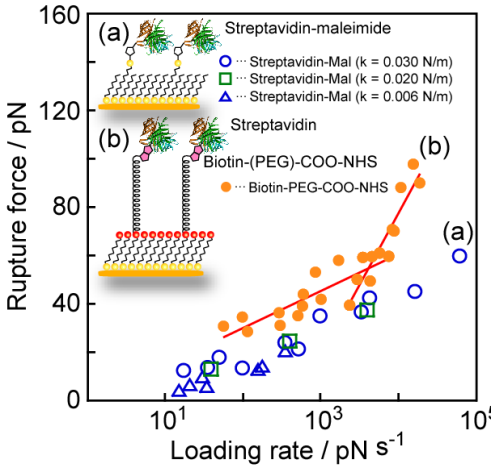

Figure 1 The most frequent rupture forces plotted against the loading rates.

References

[1] R. Merkel, et al., *Nature* 397, (1999), 50.

[2] O. Takeuchi, et al., *J. Appl. Phys.* **2006**, *100*, 074315.